Ghatti Gum-Toxicity & Teratogenicity Studies in Avian Embryos-FDA Contract #72-345 No Date

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GHATTI GUM

TOXICITY and TERATOGENICITY STUDIES in Avian Embryos

FDA Contract #72-345

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STUDIES on the TOXICITY and TERATOGENICITY of GHATTI GUM in AVIAN EMBRYOS

SUMMARY and CONCLUSIONS

Ghatti gum failed to produce a significant increase in chicken mortality under the conditions of these studies. The maximum dose level employed was 40 mg/kg (2 mg/egg).

This substance produced a significant increase in abnormal embryos at only the 9 mg/kg dose level when administered in the yolk after 96 hours incubation. Excluding those embryos showing a toxic response, in this instance hemorrhage and edema, the incidence of head-skeletal-visceral-limb abnormalities was not significantly different for any of the dose levels employed in the four test protocols.

The results of these studies indicate that ghatti gum was nontoxic and non-teratogenic in chicken embryos.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #72-345 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (pre-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Ghatti gum was solubilized in 0.12N HCl for use in the test protocols. The maximum dose level employed was 40 mg/kg (2 mg/egg). The solvent control employed in these studies consisted of the 0.12N HCl solution neutralized to pH 7.0 with sodium hydroxide. The ghatti gum solution was found to be neutral in the concentrations employed.

MORTALITY

Mortality data are shown in Tables 1 - 4. At the dose levels employed, ghatti gum was relatively nontoxic to chicken embryos. Chi-square analyses of the mortality data failed to indicate a significant difference between the solvent control groups and those receiving ghatti gum in any of the 4 test protocols (Table 5). Additional statistical analyses involving linear regression of the log dose against probit of mortality indicated a nonsignificant linear relationship between parameters (Table 6).

The results of these studies indicated that ghatti gum was nontoxic to chicken embryos under the conditions employed in these studies.

TERATOLOGY

The occurrences of abnormal embryos and those showing H-S-V-L abnormalities are shown in Tables 1 - 4. Chi-square analyses of these data indicate a significant increase in abnormalities only in those groups receiving 8 mg/kg administered in the yolk after 96 hours incubation (Table 7). Probit analyses of these data failed to yield significant linear regression between log dose and probit of abnormality incidence (Table 8).

The incidence of H-S-V-L abnormalities were not significantly different from the solvent controls for any of the dose levels in any of the four test protocols (Table 9).

The specific teratology findings associated with ghatti gum administration and with the various controls are shown in Table 10.

TABLE 1

GHATTI GUM
in 0.12N HCl

Air Cell - 0 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
40.0	156	21.15	33	1.28	2	1.92	3	0.64	1			1.28	2								
24.0	120	13.33	16	0.00	0	0.00	0														
16.0	117	20.51	24	0.85	1	0.85	1	0.85	1												
8.0	119	12.60	15	2.52	3	1.68	2	1.68	2											0.84	1
1.6	117	16.23	19	0.85	1	0.85	1	0.85	1												
0.0	97	19.58	19	0.00	0	0.00	0														
drilled	119	14.28	17	0.00	0	0.00	0														
untreated	327	10.09	33	0.61	2	0.30	1	0.30	1							0.30	1				

SUMMARY - ALL DOSE LEVELS

629	17.00	107	1.11	7	1.11	7	0.79	5			0.32	2							0.16	1

TABLE 2

GHATTI GUM
in 0.12N HCl

Air Cell - 96 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
40.0	147	15.64	23	0.68	1	0.68	1	0.68	1												
24.0	109	11.92	13	0.91	1	0.91	1					0.91	1								
16.0	109	16.51	18	2.75	3	1.83	2	0.91	1			0.91	1			0.91	1				
8.0	107	19.62	21	3.73	4	4.67	5	2.80	3			1.86	2								
1.6	110	22.72	25	1.81	2	1.81	2	0.90	1			0.90	1								
0.0	147	14.96	22	1.36	2	0.68	1					0.68	1					0.68	1		
drilled	127	15.74	20	2.36	3	2.36	3	0.78	1			0.78	1	0.78	1						
untreated	327	10.09	33	0.61	2	0.30	1	0.30	1							0.30	1				

SUMMARY - ALL DOSE LEVELS

582	17.18	100	1.89	11	1.89	11	1.03	6			0.86	5			0.17	1				

TABLE 3

GHATTI GUM
in 0.12N HCl

Yolk - 0 hrs

Dose, ppm	No. Fertile	Mortality		Abnormal				Abnormalities by category											
				Total		H-S-V-L		Head		Skeletal		Viscera		Limbs		Struc-tural		Toxic Response		Functional	
		%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#
40.0	133	43.60	58	1.50	2	1.50	2	0.75	1			0.75	1								
24.0	100	39.00	39	1.00	1	1.00	1	1.00	1												
16.0	99	42.42	42	8.08	8	3.03	3	3.03	3							1.01	1	2.02	2	2.02	2
8.0	97	32.98	32	1.03	1	3.09	3	1.03	1			1.03	1	1.03	1						
1.6	100	36.00	36	2.00	2	1.00	1							1.00	1	1.00	1				
0.0	157	33.12	52	2.54	4	1.91	3	1.27	2			0.63	1			0.63	1	0.63	1		
pierced	20	35.00	7	0.00	0	0.00	0														
untreated	327	10.09	33	0.61	2	0.30	1	0.30	1							0.30	1				

SUMMARY - ALL DOSE LEVELS

529	39.13	207	2.65	14	1.89	10	1.13	6			0.38	2	0.38	2	0.38	2	0.38	2	0.38	2

TABLE 4

GHATTI GUM
in 0.12N HCl

Yolk - 96 hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
40.0	147	30.61	45	2.04	3	1.36	2	1.36	2							0.68	1				
24.0	109	22.01	24	3.66	4	3.66	4	3.66	4												
16.0	109	19.26	21	3.66	4	4.58	5	2.75	3			0.91	1	0.91	1	0.91	1				
8.0	107	24.29	26	6.54	7	3.73	4	2.80	3					0.93	1	0.93	1	1.86	2		
1.6	108	31.48	34	3.70	4	2.77	3	1.85	2			0.92	1			0.92	1				
0.0	147	24.48	36	0.68	1	0.68	1	0.68	1							0.68	1				
pierced	126	30.95	39	0.79	1	0.79	1	0.79	1												
untreated	327	10.09	33	0.61	2	0.30	1	0.30	1							0.30	1				

SUMMARY - ALL DOSE LEVELS

580	25.86	150	3.79	22	3.10	18	2.41	14			0.34	2	0.34	2	0.69	4	0.34	2		

TABLE 5

GHATTI GUM

Chi-Square Analyses of Mortality

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1.600	0.210	2.043	0.115	1.197
8.000	1.473	0.654	0.013	0.013
16.000	0.000	0.027	1.879	0.708
24.000	1.123	0.266	0.684	0.098
40.000	0.020	0.000	2.933	1.091
All Doses (DF)	6.047(5)	5.744(5)	5.427(5)	7.123(5)

TABLE 6

GHATTI GUM

Probit Analyses of Mortality

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
LD-30	NS	NS	NS	NS
LD-50	NS	NS	NS	NS
LD-70	NS	NS	NS	NS

TABLE 7

GHATTI GUM

Chi-Square Analyses of Abnormalities

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1.600	0.009	0.047	0.020	1.597
8.00	0.981	0.662	0.145	5.186*
16.00	0.009	0.115	3.014	1.568
24.000	0.000	0.068	0.170	1.568
40.000	0.152	0.000	0.043	0.253
All Doses (DF)	5.328(5)	4.558(5)	14.342(5)	7.836(5)

*Probability $<$ 0.05

TABLE 8

GHATTI GUM
Probit Analyses - Abnormalities

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
	NS	NS	NS	NS

TABLE 9

GHATTI GUM

Chi-Square Analyses of HLSV Abnormalities

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1.600	0.009	0.064	0.003	0.676
8.000	0.323	1.625	0.001	1.625
16.000	0.009	0.068	0.023	1.568
24.000	0.000	0.255	0.003	1.568
40.000	0.152	0.503	0.035	0.000
All Doses (DF)	3.265(5)	5.342(5)	2.043(5)	4.754(5)

TABLE 10

GHATTI GUM

TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Control		327	2	1	dwarfism
				1	dysgnathia - beak
Drilled - 96 hrs		127	3	1	abnormal curvature - toe
				1	celosomia - abdomen
				1	exencephaly; abnormal shortening - maxilla
Pierced - 96 hrs		126	1	1	dysgnathia - beak
Air Cell - 0 hrs	40.0 mg/kg	156	2	1	fusion failure - abdomen
				1	agenesis - head; displacement - maxilla;
					celosomia - abdomen
	16.0	117	1	1	anophthalmia
	8.0	119	3	1	cachexia
				1 1	anophthalmia; exencephaly; dysgnathia - beak anophthalmia; dysgnathia - beak
	1.6	117	1	1	anophthalmia; exencephaly; abnormal shortening -maxilla
Air Cell - 96 hrs	40.0	147	1	1	anophthalmia; dysgnathia - beak
	24.0	109	1	1	fusion failure -abdomen
	16.0	109	3	1	anophthalmia; agenesis - maxilla
				1	fusion failure - abdomen
				1	dwarfism

TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Air Cell - 96 hrs	8.0 mg/kg	107	4	1	anophthalmia; acrania; agenesis - maxilla
				1	celosomia - abdomen
				1	agenesis - eyelid
				1	exencephaly; celosomia - abdomen
	1.6	110	2	1	celosomia - abdomen
				1	exencephaly
	0.0	147	2	1	celosomia - abdomen
				1	hemorrhage
Yolk - 0 hrs	40.0	133	2	1	celosomia - abdomen
				1	anophthalmia; dysgnathia - beak
	24.0	100	1	1	exencephaly
	16.0	99	8	2	cachexia
				1	hemorrhage
				1	exencephaly
				2	anophthalmia; dysgnathia - beak
				1	dwarfism
				1	umbilical cord around neck
	8.0	97	1	1	anophthalmia; exencephaly; abnormal shortening - maxilla; dysgnathia - beak; agenesis - wing; agenesis - hindlimb; celosomia - abdomen

TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Yolk - 0 hrs	1.6 mg/kg	100	2	1	malrotation - hindlimb
				1	dwarfism
	0.0	157	4	1	celosomia - abdomen
				1	anophthalmia; exencephaly; dysgnathia - beak
				1	umbilical cord around neck
				1	anophthalmia; exencephaly; microcephaly; dysgnathia - beak; dwarfism
Yolk - 96 hrs	40.0	147	3	1	dwarfism
				2	anophthalmia; exencephaly; dysgnathia - beak
	24.0	109	4	1	buphthalmia
				1	anophthalmia; dysgnathia - beak
				2	exencephaly
	16.0	109	4	1	fusion failure - abdomen
				1	anophthalmia; agenesis - eyelid; dysgnathia - beak
				1	anophthalmia; buyphthalmia; agenesis - mandible and hindlimb; dwarfism
				1	anophthalmia; dysgnathia - beak

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Yolk - 96 hrs 8.0 mg/kg	107	7	2	exencephaly
			1	agenesis - down
			1	anbormal curvature - toe
			1	hemorrhage
			1	buphthalmia; dysgnathia - beak
			1	edema
1.6	108	4	1	celosomia - abdomen
			2	exencephaly
			1	dwarfism
0.0	147	1	1	anophthalmia; agenesis - maxilla; dysgnathia - beak; dwarfism